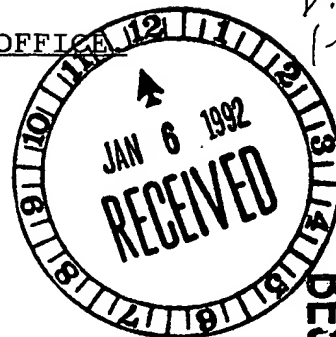




THE UNITED STATES PATENT AND TRADEMARK OFFICE



#12
B. White
1-23-92

In re Application of Keith)
Serial Number: 07/542,149) Art Unit: 1814
Filed: June 22, 1990) Examiner: C. Low

For: PERTUSSIS TOXIN GENE: CLONING AND EXPRESSION

DECLARATION UNDER 37 CFR 1.131

Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

I, Jerry M. Keith am named as the inventor in the above indicated patent application, and I state as follows:

1. That prior to July 1, 1988, a cloned gene coding for the expression of a mutant *Bordetella pertussis* toxin polypeptide fragment having substantially reduced enzymatic activity associated with pertussis toxin reactogenicity and retaining an epitope reactive with monoclonal antibody 1B7 was conceived and reduced to practice. This cloned gene and its expression product have the laboratory designation mutant 4-1. Mutant 4-1 possesses and exhibits the characteristics disclosed in Patent applications 07/311,612 and its continuation 07/542,149.

2. Exhibit pages 1-6 include laboratory notebook pages which demonstrate the reactivity of mutant 4-1 to monoclonal antibody 1B7. The data from these pages resulted in Figure 6B in Patent applications 07/311,612 and its continuation 07/542,149, and Figure 1B in Burnette et al. Science 242:72-74 (October 1988).

BEST AVAILABLE COPY

3. Exhibit pages 7-15 include laboratory notebook pages which demonstrate ADP-ribosyltransferase assays involving various pertussis toxin mutants, including a demonstration of substantially reduced enzyme activity associated with mutant 4-1. The data from these pages resulted in Figure 2A described in Burnette et al. Science 242:72-74 (October 1988), and the corresponding panel in Figure 6 of Patent applications 07/311,612 and its continuation 07/542,149.

4. Exhibit pages 16-25 include laboratory notebook pages which demonstrate NAD-glycohydrolase assays involving various pertussis toxin mutants, including a demonstration of substantially reduced enzyme activity associated with mutant 4-1. The data from these pages resulted in Figure 2B described in Burnette et al. Science 242:72-74 (October 1988), and the corresponding panel in Figure 6 of Patent applications 07/311,612 and its continuation 07/542,149.

5. The actual dates on laboratory notebook pages described in sections 2-4 above have been blocked out. I state that each laboratory notebook page in sections 2-4 above was dated prior to July 1, 1988.

6. The work corresponding to sections 1-5 above was carried out in the United States, and under my direction.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and

belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date Dec 19, 1991

Jerry M. Keith

Jerry M. Keith

EXHIBIT PAGES 1-25

EXHIBIT PAGE #1

Western blots - Aragen nutrients;

for stained gel - using solubilized inclusion bodies

Lane

- 1 Blank
- 2 Bio-Rad stds
- 3 PTx (5ug)
- 4 6A
- 5 1-1 5-1
- 6 2-2 4-1
- 7 3-3 3-1
- 8 4-4 2-2
- 9 5-5 1-1
- 10 6-6 2-1
- 11 7-7 7-2
- 12 8-8 6-1
- 13 Blank 6A-2
- 14 Blank
- 15 Blank

5ug of inclusion body prep;
= 10ul/well;

probe w/ 187

Western

Pattern gel
used the gel for protein

Lane

- 1 Blank
- 2 RS markers (row)
- 3 Blank
- 4 PTx (1ug) + up this
- 5 6A
- 6 1-1 5-1
- 7 2-2 4-1
- 8 3-3 3-1
- 9 4-4 2-1
- 10 5-5 1-1
- 11 6-6 8-1
- 12 7-7 7-2
- 13 8-8 6-1
- 14 Blank 6A-2
- 15 Blank

500ug of 15ug protein in 50% glycerol;

6-test equivalents
6-test 6x2 format;

12/17/91
SENT BY:

18:16

330 02 0396

NIDR/LME

12-17-91 : 2:31PM :

32-

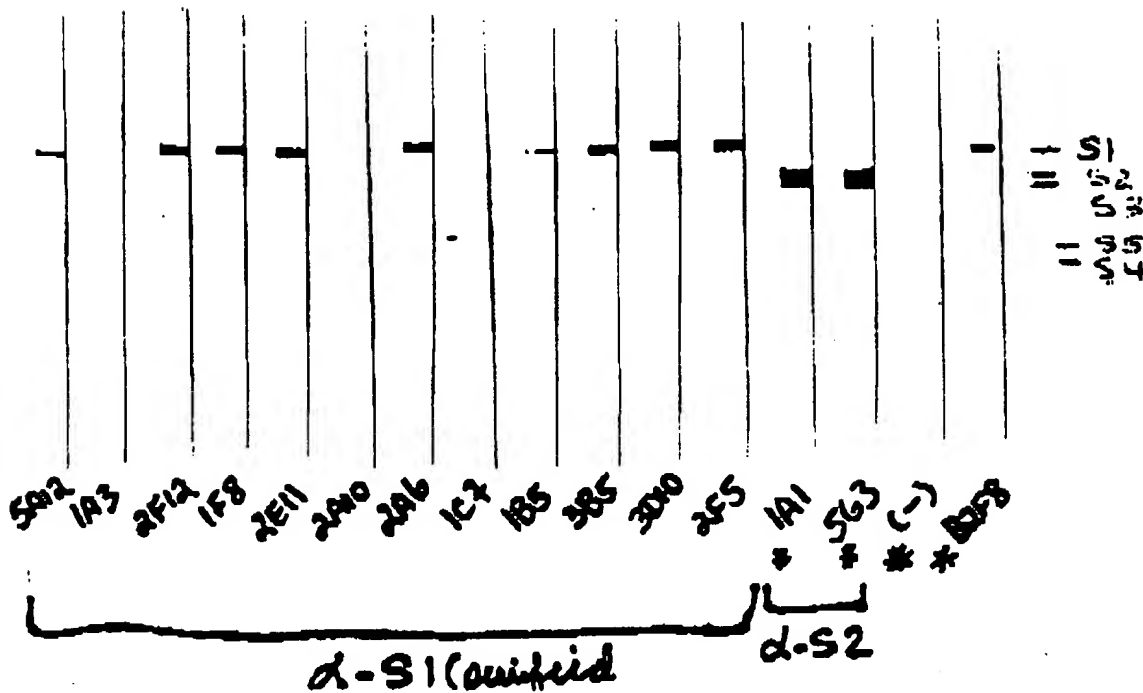
301 402 0396:# 4

004

EXHIBIT PAGE #2

PTX

10ug



PTX 1A3 4-1 3-1 2-2 1-1 6-1 7-2 4-1 5-1 4-4

--✓-----=

Amgen mutant S1 proteins
monoclonal 1B7

12/17/91

18:16

33

402 0396

NIDR/LME

003

SENT BY:

12-17-91 : 2:32PM :

32-

301 402 0396: # 5

EXHIBIT PAGE #3

Take samples (Ammonia) - dilute 1:1 w/ my buffer

Run ~20ul for protein (5ug)

~10ul for water (~2.5ug total);

Protein

Water

Lane

- 1 Blank
- 2 10ul Bro-East Std
- 3 PTx (5ul) = 5ug
- 4 6A
- 5 5-1
- 6 4-1
- 7 3-1
- 8 2-1
- 9 1-1
- 10 8-1
- 11 7-2
- 12 10-1
- 13 6A-2
- 14
- 15 B2-2

Blank

PRL Std (5ul)

PTx (2.5ul)

Same amount use 10ul

187

12/17/91
SENT BY:

18:17

301 402 0396

NIDR/LME

12-17-91 : 2:34PM :

32-

301 402 0396: # 8

EXHIBIT PAGE #4

Protein Gel - Arrows nutrients in missouri books
- 10ml of 500mg/ml in 1% Lucernum
in 5mg ea (Sent by Akal)

Next time cut down on 1-1 abit for protein gel;

PTX (GND)
1-1 5-1 4-1 3-1 2-1 1-1 9-1 7-2 6-1 4-2
pt. protein gel

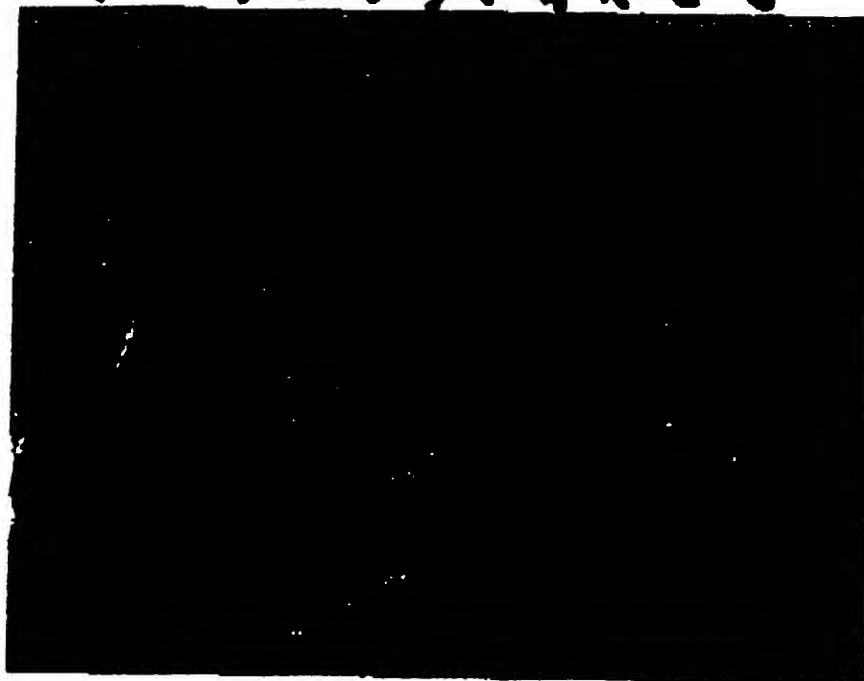


EXHIBIT PAGE #5

For Western B(w/ new machine) and Protein

For 200ul of 1mg/ml in Lysate				
DTX (1ug)	Sample	2x	1x	Need
pu C18	52.2	52.2	95.5	78.4 78.4 43.1
S13	28.9	28.9	112	- remove
A528	33.7	33.7	132.5	too low
hys 9	39.6	39.6	120.7	
Nis 9	51.5	51.5	96.9	↓ actually
Ala 9	58.1	58.1	83.7	0.36
Del 9	56.6	56.6	26.8	amc
Nis 8:9	48.0	48.0	103.8	
hys 58	44.5	44.5	110.9	
Cly 41	33.1	33.1	133.7	
Sch 41	27.2	27.2	145.5	
Del 41	33.3	33.3	133.3	

Made 200ul samples of (C) at 1mg/ml in Lysate,

loaded (20ul = 20ug) into each lane of gel -

1 strip

1 blot,

(Expt about pTX13 (in the moment))

	Sample	2x	1x
removed: New pS13 -	28.43	78.43	43.1

Blotted 2 gels - picked w/ 2F12 and 1B7

12/17/81
SENT BY:

18:18

30

02 0396

NIDR/LME

: 12-17-81 : 2:37PM :

32-

301 402 0386:# 8

008

EXHIBIT PAGE #6

(Handwritten numbers)

137

2F124

EXHIBIT PAGE #7

ADP-ribosyl transferase H5090 - Argeri mutants -
and S13 mutants; Hg new transducer (glycerol)

		FOR	Sup/ml	buffer
	Stock (SA)			
20ug/20ul	purified S1	400	12.5	987.5
	6A	225	22.2	977.8
	7-1	218	22.9	977.1
	2-2	239	20.9	979.1
	3-1	267	18.7	981.3
	4-1	247	20.2	979.8
	5-1	156	32.0	968.8
	6-1	126	39.7	960.3
	7-2	183	27.3	972.7
	8-1	135	37.0	963.8
	20-A	230 (total)	30.0	970.0
	S11-4	199	25.1	975.8
20ug/20ul	plc	1.34	0.373	0.172
	S13	2.55	0.196	0.305
	lys 58	4.49	0.111	0.389
	His 8-9	4.16	0.120	0.380
	del 9	2.31	0.216	0.281
	his 9	3.88	0.128	0.372
	ala 9	3.44	0.145	0.355

0.5mg/1mg/ml

0.5 hr, 37°C

* Mutants prepared from new His- finger (stock) - no glycerol;

EXHIBIT PAGE # 8

SAM

1	369.70	475.10	21345.40	10.00	
2	442.30	7258.80	22540.50	10.00	
3	57.30	5555.60	20702.30	10.00	
4	427.40	5544.00	24382.60	10.00	
5	450.80	5594.30	25768.10	10.00	262 ✓
6	449.30	5592.00	26200.70	10.00	
7	130.70	1877.50	5925.50	10.00	
8	163.50	2097.50	8630.70	10.00	31-1 ✓
9	137.80	1565.50	7625.40	10.00	
10	367.60	5671.50	20435.10	10.00	
11	396.20	5078.90	24578.20	10.00	42-2 ✓
12	371.60	5389.20	21945.60	10.00	
13	196.10	2095.20	12523.20	10.00	
14	287.20	4389.90	15388.10	10.00	53-1 ✓
15	234.30	3437.70	12737.90	10.00	
16	34.20	211.90	748.00	10.00	
17	32.90	187.00	754.60	10.00	64-1 ✓
18	35.00	223.20	762.40	10.00	
19	400.60	4613.40	27887.60	10.00	
20	379.10	4982.40	25621.90	10.00	75-1 ✓
21	446.20	6774.50	27576.10	10.00	
22	38.40	228.60	896.20	10.00	
23	31.40	219.80	749.50	10.00	86 ✓
24	31.70	178.80	649.30	10.00	
25	33.30	170.70	729.40	10.00	
26	32.00	178.20	745.60	10.00	97-2 ✓
27	33.10	184.70	797.40	10.00	
28	31.90	155.30	696.20	10.00	
29	39.00	268.20	1092.50	10.00	108 ✓
30	35.90	208.60	992.40	10.00	
31	35.50	218.30	918.40	10.00	
32	25.20	249.50	2089.70	4.50	recount 792 1120A →
33	35.20	195.20	809.90	10.00	
34	37.70	257.30	947.30	10.00	
35			948.00	10.00	
36			963.00	10.00	

EXHIBIT PAGE #9

Construct (100ng) cpm \pm SD *

upper limit

6A 25450 \pm 950

26400

1-1 7393 \pm 1367

8760

2-2 22319 \pm 2096

24415

3-1 13549 \pm 1596

~~704~~ 15145

4-1 754 \pm 7

761

5-1 26361 \pm 1321

27682

6-1 764 \pm 124

868

7-2 753 \pm 30

783

8-1 926 \pm 205

1131

first 20A 839 \pm 68

907

RSI/1-4 952 \pm 9

961

* S.A. of 32P-NAD may have been a little on the low end

EXHIBIT PAGE #. 10

ADP-Ribosyltransferase Activity

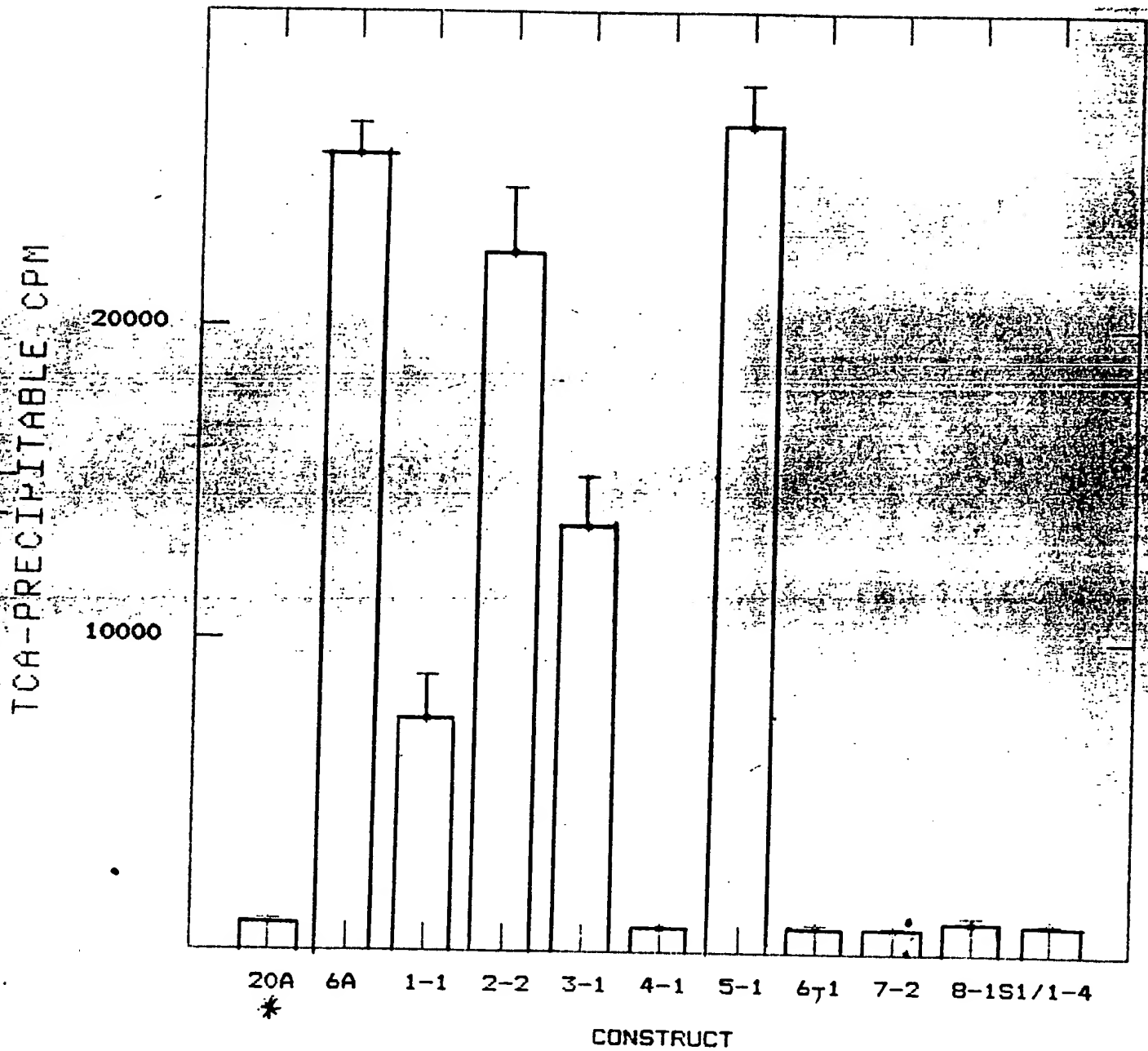
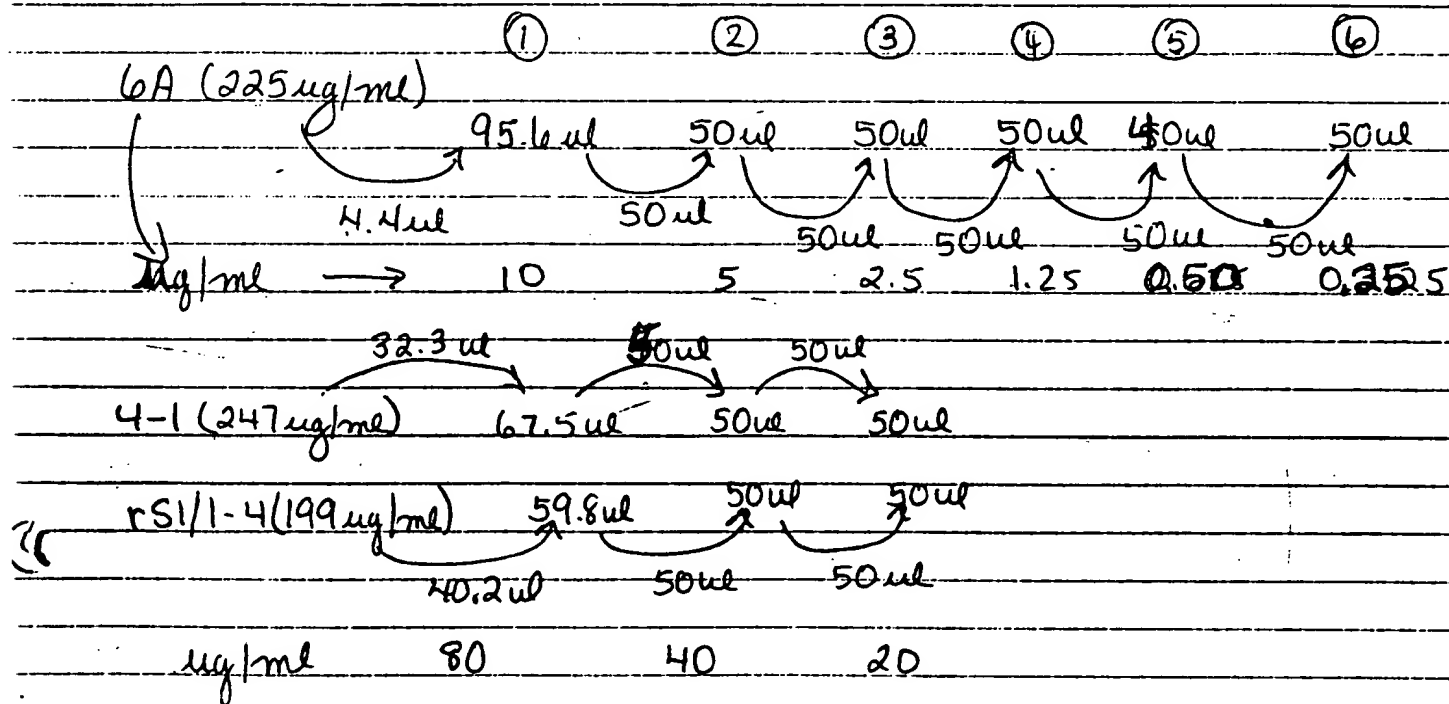


EXHIBIT PAGE #11

ADP-ribosyl transferase activity: 20A, 6A, 4-1 and SI/1-4

- 1) All stocks frozen in 50 mM Tris HCl, pH 8.0 protein and densitometric scans already performed
- 2) Dilutions for assay: use Tris buffer:



- 3) Use 20 ul of each prep in 40 ul assay; assay 30' at 37°C w/ 4 ug/T;

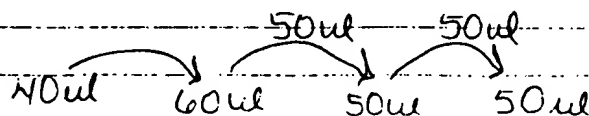
4) Final concentrations: (ug/ml)

6A 0.185, 0.3125, 0.625, 1.25, 2.5, 5.

4-1 } 10, 20, 40
rSI/1-4 }

- 5) Reaction mixtures: 20 ul diluted
10 ul 4X cocktail
10 ul Transducin (400 ug/ml 50% glycerol)

N.B. 20A dilute



35C

1.1M

EXHIBIT PAGE # 12

PAGE:

() 1 TO SURVEY PRESET TIME: 100
 () 1 REPEAT: 1 CYCLE REPEAT: 1 SCRN RS232IN
 1 0 ACC IN GCF IN ROM IN
 CHANNEL 1-LL: 1L: 400 ZSIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 1
 CHANNEL 2-LL: 2L: 670 ZSIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 2
 CHANNEL 3-LL: 3L: 1000 ZSIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 3
 DATA CALC: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR: 0.00000
 HALF LIFE (DAYS): IN

SAM	CPM1	CPM2	CPM3	TIME	
1	28.00	159.00	574.00	1.00	> Blank (46)
2	31.00	166.00	558.00	1.00	
3	754.00	11693.00	47197.00	1.00	> 6A 200ng 45649
4	769.00	10012.00	45333.00	1.00	
5	723.00	12202.00	38958.00	1.00	> " 100ng 39883
6	728.00	8698.00	42031.00	1.00	
7	336.00	9170.00	35608.00	1.00	> " 50ng 37581
8	759.00	11763.00	40786.00	1.00	
9	330.00	4358.00	21631.00	1.00	> " 25ng 26686
10	670.00	9409.00	32971.00	1.00	
11	256.00	3685.00	14666.00	1.00	> " 10ng 13464
12	177.00	2114.00	13494.00	1.00	
13	168.00	1974.00	9214.00	1.00	> " 5ng 7588
14	154.00	1863.00	7195.00	1.00	
15	33.00	151.00	664.00	1.00	> 20A 1.6ug 39
16	32.00	169.00	646.00	1.00	
17	32.00	190.00	713.00	1.00	> " 0.8ug 92.5
18	37.00	144.00	538.00	1.00	
19	38.00	151.00	711.00	1.00	> " 0.4ug 102.5
20	39.00	191.00	726.00	1.00	
21	46.00	194.00	745.00	1.00	
22	40.00	205.00	738.00	1.00	> 4-1 1.6ug 149.5
23	45.00	241.00	800.00	1.00	
24	34.00	203.00	740.00	1.00	> " 0.8ug 219
25	36.00	195.00	672.00	1.00	
26	34.00	200.00	585.00	1.00	> " 0.4ug 62.5
27	36.00	410.00	1464.00	1.00	
28	48.00	466.00	1704.00	1.00	> SI/1-4 1.6ug 268
29	48.00	263.00	589.00	1.00	
30	47.00	246.00	656.00	1.00	> " 0.8ug 406.5
31	47.00	241.00	654.00	1.00	
32	47.00	245.00	602.00	1.00	> " 0.4ug 312

$\bar{x} = 78$

compared to buffer

% Control

4-1 max = 0.01 % > 6000-fold decrease

SI/1-4 = 0.03 % > 2,000-fold decrease

NE

4/4

EXHIBIT PAGE # 13

Sample	Protein [ug/ml]	X cpm	Net cpm
--------	--------------------	----------	------------

Buffer	10.0 -	616	-
2A	0.125	8205	7589
"	0.250	14080	13464 *
"	0.625	27301	26685
"	1.25	38197	37581
"	2.5	42150	41534
"	5.0	46265	45649

20A	10.0 eqw	708	} x = 78 cpm/ug
"	20.0 eq	655	
"	40 eqw		

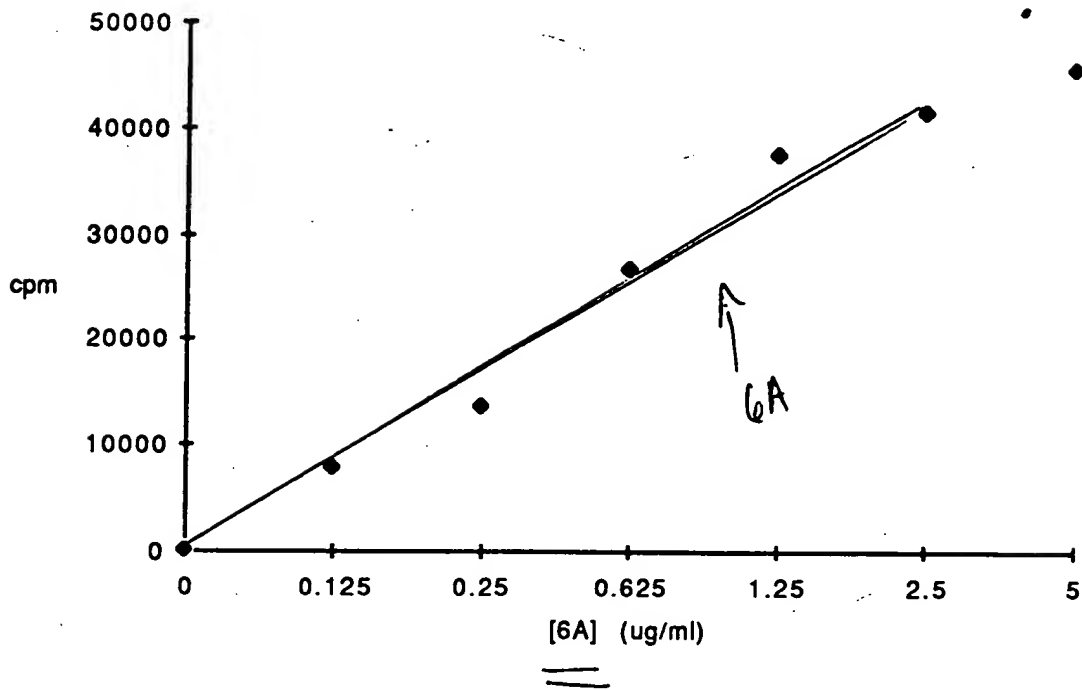
				red factor
4-1	10	679	-39	>5000
"	20	835	127	8481
"	40	765	110	>10,000

SI/1-4	10	928 928	210	2564
"	20	1023 1023	315	3419
"	40	928 1584	929	2318

these refer to processed protein content

EXHIBIT PAGE # 14

Series 4:22:47 PM



ADP-Ribosylation of Transducin

EXHIBIT PAGE #15

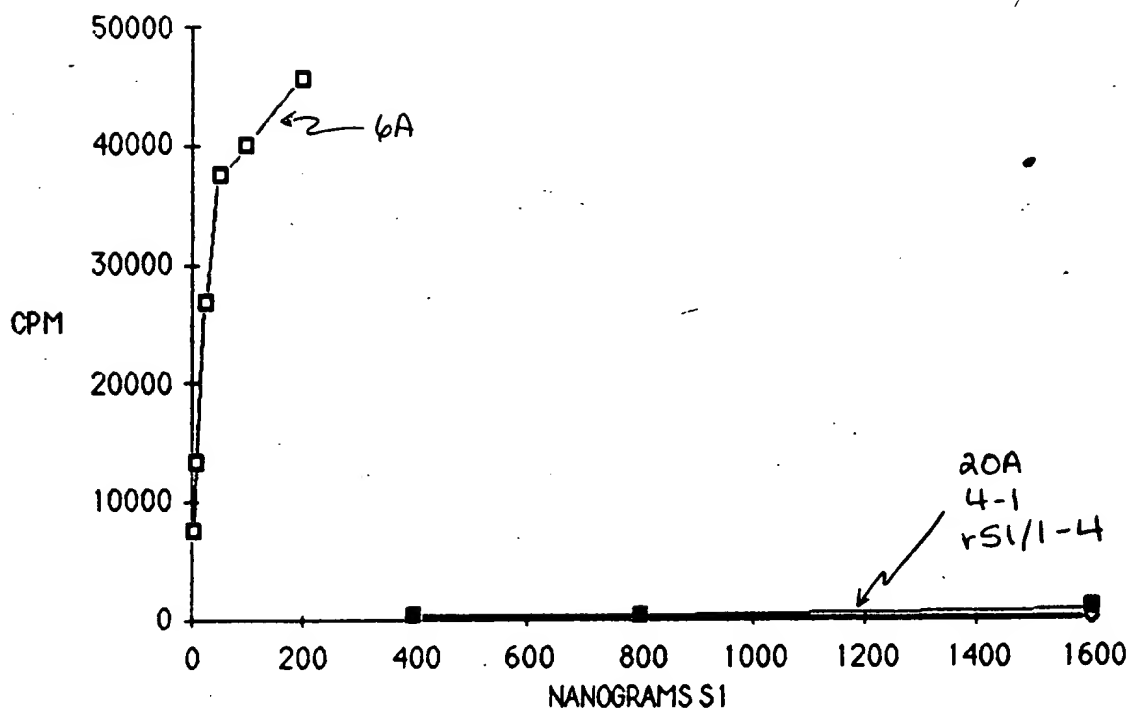


EXHIBIT PAGE # 16

VAD glycohydrolase assay

40 ug/ml in 100ul

6A	225	17.7	82.2
35A	69	57.9	42.1
39A	88	45.4	54.5
33B	113	35.3	64.7
2B	125	32.0	68.0
3B	157	25.4	74.6
1-1	75	53.3	46.6
2-2	75	↓	↓
3-1	75		
4-1	75		
5-1	75		
6-1	75		
7-2	75		
8-1	75		
20A	753	↓	↓

Assayed standard protein in duplicate; 30°C for 4 hours;

50 ug/ml = 1 ug/100

$$\text{Cpm} \times 1.5 \times 1.5 \div 120 \div 76.9 \xrightarrow{\text{1 pmole}}$$

EXHIBIT PAGE # 17

NAD glycohydrolase - used 20A glycol control
all at 1ug/assay

% control \pm S.D. (total)

6A	100
35A	105.7 \pm 7.6
39A	35.3 \pm 2.3
33B	3.9 \pm 0.8
2B	1.6 \pm 1.5
3B	1.5 \pm 1.2

1-1	6.1 \pm 0.98
2-2	47.6 \pm 3.1
3-1	9.1 \pm 2.0
4-1	2.2 \pm 0.4
5-1	132.1 \pm 7.4
6-1	1.7 \pm 0.4
7-2	2.2 \pm 0.6
8-1	2.6 \pm 0.4

New data

CL

EXHIBIT PAGE #18

1 ug/assay

NAD glycohydrolase

20A (glycol)	805 \pm 12	Net cpm	pmols rel/min/ug	%
6A	17,310 \pm 701	16505	4.02	100
35A /	18,257 \pm 1023	17452	4.25	105
39A /	6645 \pm 304	5840	1.42	35
33B /	1452 \pm 136	647	0.15	3.7
2B /	1072 \pm 247	267	0.065	1.6
3B /	1062 \pm 184	257	0.062	1.5
20A (Trie)	1358 \pm 278	-	-	-
1-1 /	1814 \pm 156	1009	0.24	5.9
2-2 /	8670 \pm 399	7865	1.9	4.7
3-1 /	2303 \pm 329	1498	0.36	8.9
4-1 /	1175 \pm 67	372	0.09	2.2
5-1 /	22,615 \pm 796	21,810	5.3	131.8
6-1 /	1685 \pm 70	280	0.068	1.7
7-2 /	1169 \pm 102	364	0.088	2.2
8-1	1233 \pm 59	428	0.10	2.48

EXHIBIT PAGE #19

USER: 2 ID: SURVEY PRESET TIME: 1.00
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCRN: RS2P2: N
 A: 0 ACC: N DEF: N ROM: N
 CHANNEL 1-LL: 0 UL: 400 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR:
 CHANNEL 2-LL: 0 UL: 670 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR:
 CHANNEL 3-LL: 0 UL: 1000 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR:
 DATA CALC: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR: 0 1.00000
 HALF LIFE (DAYS): N

SAM	CPM1	CPM2	CPM3	TIME
1	6789.00	16577.00	16590.00	1.00
2	7141.00	17380.00	17387.00	1.00
3	7418.00	17974.00	17980.00	1.00
4	7051.00	17110.00	17117.00	1.00
5	7778.00	18524.00	18596.00	1.00
6	7847.00	19077.00	19085.00	1.00
7	2802.00	6760.00	6774.00	1.00
8	2652.00	6300.00	6316.00	1.00
9	2894.00	6874.00	6886.00	1.00
10	701.00	1566.00	1576.00	1.00
11	598.00	1488.00	1498.00	1.00
12	557.00	1302.00	1315.00	1.00
13	419.00	921.00	929.00	1.00
14	403.00	939.00	950.00	1.00
15	579.00	1357.00	1375.00	1.00
16	548.00	1275.00	1286.00	1.00
17	394.00	951.00	965.00	1.00
18	415.00	952.00	980.00	1.00
19	812.00	1994.00	1998.00	1.00
20	703.00	1729.00	1739.00	1.00
21	708	1719.00	1731.00	1.00
22	3565	9030.00	9044.00	1.00
23	3326.00	8740.00	8750.00	1.00
24	3183.00	8241.00	8249.00	1.00
25	888.00	2273.00	2294.00	1.00
26	1038.00	2546.00	2561.00	1.00
27	747.00	990.00	2001.00	1.00
28	493.00	1196.00	1205.00	1.00
29	175.00	1234.00	1240.00	1.00
30	406.00	1109.00	1119.00	1.00
31	1434.00	2171.00	2172.00	1.00
32	1997.00	23210.00	23025.00	1.00
33	1822.00	22924.00	22939.00	1.00
34	487.00	1089.00	1106.00	1.00
35	171.00	1152.00	1152.00	1.00
36	704.00	1011.00	1012.00	1.00
37	402.00	1101.00	1104.00	1.00
38	110.00	1050.00	1050.00	1.00
39	150.00	1051.00	1050.00	1.00
40	100.00	100.00	100.00	1.00
41	100.00	100.00	100.00	1.00
42	100.00	100.00	100.00	1.00
43	100.00	100.00	100.00	1.00
44	100.00	100.00	100.00	1.00
45	100.00	100.00	100.00	1.00
46	100.00	100.00	100.00	1.00
47	100.00	100.00	100.00	1.00
48	100.00	100.00	100.00	1.00
49	100.00	100.00	100.00	1.00
50	100.00	100.00	100.00	1.00

LOA(Tris): 1353 ± 278

20A (glycerol)

Need ADP-ribosyltransferase

EXHIBIT PAGE 20

NAD glycohydrolase Activity - Aringer mutants (New pipette
Tris buffer)

2 hrs, 30°C, 30 μM NAD

CPM (TOTAL)

Construct	0.25 μg	0.5 μg	1.0 μg
purified SI from PTx	6,340 (1.54)	12,980.5 (3.16)	—
20A	F28		- 23 (0)
6A	1480.5 (0.36)	3,074 (0.75)	6446 (1.57)
1-1	73.5 (0.02)	254.5 (0.06)	486.5 (0.12)
2-2	562.5 (0.14)	1340 (0.33)	2734 (0.66)
3-1	125 (0.03)	419 (0.10)	882.5 (0.21)
4-1	31.5 (0.008)	-11 (0) (0)	34.5 (0.008)
5-1	1369 (0.33)	3011 (0.73)	6204 (1.51)
6-1	-5 0	-42 0	-30.5 0
7-2	-59 0	15 0	-58 0 0.00
8-1	-5.5 0	-4 0	204 (0.05)
SI/1-4	-60 0	-99.5 0	-64 0

$$\text{cpm} \times \overset{\text{NAD}}{1.5} \times \overset{\text{vol}}{1.5} \times \overset{\text{boles}}{1.3} \div 100 \div \overset{\text{hrs}}{120} \div \mu\text{g}$$

$$= .0002437 \div \mu\text{g} = \text{pmol/min.}$$

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PAGE

SR: 2 ID: SURVEY PRESET TIME: 1.00
 FILE REPEAT: 1 CYCLE REPEAT: 1 SCRIP: 352321N
 : 0 AGC: N DCF: N RCM: N
 CHANNEL 1-LL: 0 UL: 400 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR:
 CHANNEL 2-LL: 0 UL: 670 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR:
 CHANNEL 3-LL: 0 UL: 1000 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR:
 DATA CALC: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR: 0 1.00000
 HALF LIFE (DAYS): N

SAM	CPM1	CPM2	CPM3	TIME
1	253.00	633.00	629.00	1.00 > Bulk
2	301.00	761.00	773.00	1.00
3	429.00	3446.00	3453.00	1.00
4	497.00	3564.00	3579.00	1.00
5	5456.00	13055.00	13062.00	1.00 > 61 0.5
6	5969.00	14300.00	14314.00	1.00
7	2780.00	6637.00	6647.00	1.00 > " 0.25
8	3170.00	7437.00	7452.00	1.00
9	2777.00	7116.00	7122.00	1.00 > 6A 1.0
10	2851.00	7170.00	7178.00	1.00
11	1510.00	3808.00	3822.00	1.00 > " 0.5
12	1553.00	3734.00	3745.00	1.00
13	956.00	2258.00	2266.00	1.00 > " 0.25
14	879.00	2097.00	2104.00	1.00
15	492.00	1096.00	1104.00	1.00 > 1-1 1.0
16	523.00	1271.00	1286.00	1.00
17	436.00	978.00	991.00	1.00 > " 0.5
18	344.00	925.00	933.00	1.00
19	322.00	760.00	778.00	1.00 > " 0.25
20	315.00	781.00	791.00	1.00
21	1299.00	3243.00	3250.00	1.00 > 2-2 1.0
22	1449.00	3619.00	3630.00	1.00
23	802.00	1999.00	2009.00	1.00 > " 1.0
24	819.00	2075.00	2082.00	1.00
25	518.00	1255.00	1272.00	1.00 > " 1.0
26	548.00	1264.00	1272.00	1.00
27	644.00	1631.00	1645.00	1.00 > 3-1 1.0
28	636.00	1526.00	1536.00	1.00
29	466.00	1060.00	1076.00	1.00 > " 0.5
30	516.00	1172.00	1181.00	1.00
31	339.00	829.00	841.00	1.00 > " 0.25
32	338.00	815.00	826.00	1.00
33	304.00	734.00	750.00	1.00 > 4-1 1.0
34	339.00	729.00	738.00	1.00
35	310.00	679.00	684.00	1.00 > " 0.5
36	265.00	596.00	608.00	1.00
37	330.00	735.00	745.00	1.00 > " 0.25
38	294.00	722.00	732.00	1.00
39	2549.00	6613.00	6625.00	1.00 > 5-1 1.0
40	2418.00	7169.00	7204.00	1.00
41	121.00	1719.00	1728.00	1.00 > " 0.5
42	243.00	1697.00	1732.00	1.00
43	110.00	2009.00	2022.00	1.00 > " 0.25
44	123.00	1723.00	1733.00	1.00

40

EXHIBIT PAGE 22

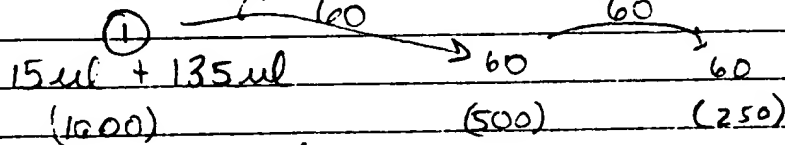
CPM1	CPM2	CPM3	TIME
251.00	551.00	559.00	1.00 > 6-1 1.0
276.00	582.00	533.00	1.00
254.00	551.00	567.00	1.00 > " 0.5
270.00	559.00	559.00	1.00
302.00	710.00	720.00	1.00 > " 0.25
287.00	574.00	586.00	1.00
286.00	557.00	569.00	1.00 > 7-2 1.0
295.00	721.00	729.00	1.00
288.00	599.00	709.00	1.00 > " 0.5
317.00	725.00	733.00	1.00
240.00	613.00	524.00	1.00 > " 0.25
273.00	563.00	577.00	1.00
359.00	656.00	870.00	1.00 > 8-1 1.0
412.00	946.00	957.00	1.00
284.00	588.00	596.00	1.00 > " 0.5
299.00	598.00	710.00	1.00
272.00	666.00	671.00	1.00 > " 0.25
277.00	717.00	727.00	1.00
290.00	652.00	658.00	1.00 > VS11-4 1.0
245.00	614.00	621.00	1.00
207.00	569.00	577.00	1.00 > " 0.5
258.00	626.00	534.00	1.00 > " 0.25
259.00	582.00	535.00	1.00
302.00	592.00	700.00	1.00
290.00	579.00	688.00	1.00 > 20A 1.0 equiv.*
265.00	563.00	676.00	1.00

EXHIBIT PAGE 23

NAD glycohydrolase Activity Assay nutrients, ^(duplicate) ~~(triplicate)~~
at 250, 500 and 1000 ng 2 hours 30°C;

PSI (4100 ug/ml)

dilute to 410 ug/ml in 1:10 and 50 ul for ^(cc) pt;



Nutrits: at 75 ug/ml

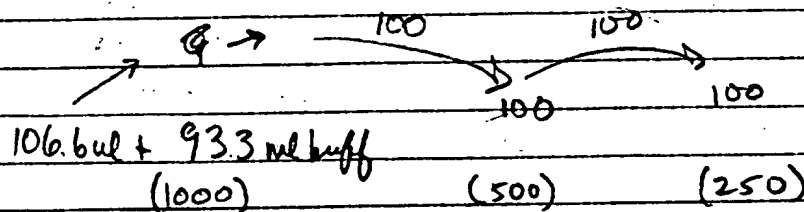
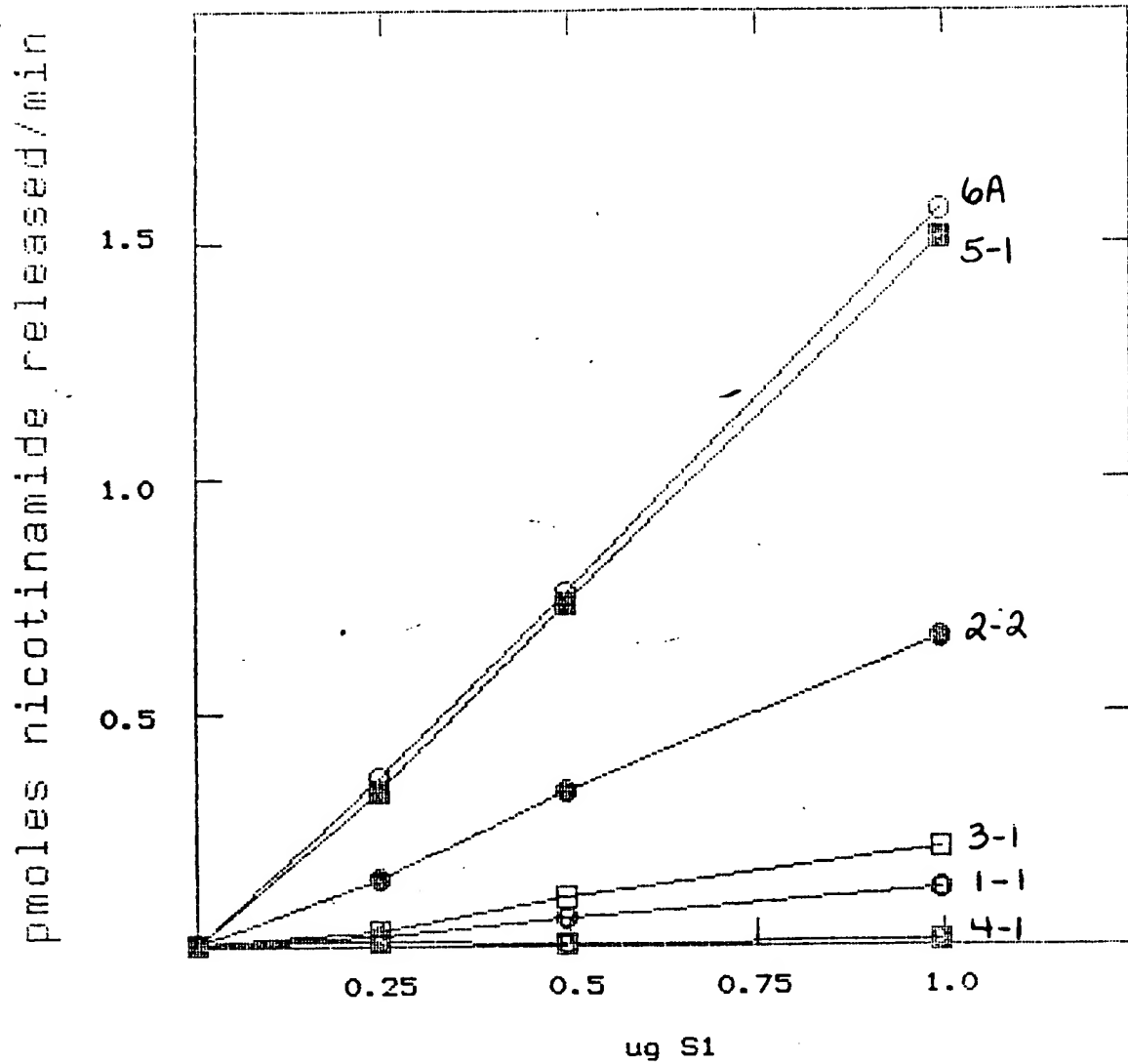


EXHIBIT PAGE 24

	<u>0.25</u>	<u>0.5</u>	<u>1.0</u>	<u>% control</u>
1A	0.36	0.75	1.57	100
1-1	0.02	0.06	0.12	7.6
2-2	0.14	0.33	0.66	42.0
3-1	0.03	0.10	0.21	13.3
4-1	0.008	0.0	0.008	0.51
5-1	0.33	0.73	1.51	96.7
6-1		0	0	0
7-2		0	0.05	3.1
8-1		0	0	0

EXHIBIT PAGE # 25



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